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In Vitro Evaluation of the Antibacterial Activity of Hydroethanolic Extracts of Four Medicinal Plants from Côte d'Ivoire

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ABSTRACT

The antibacterial activities of hydromethanolic extracts of *Margaritaria discoidea* and *Parkia biglobosa* trunk barks, *Trichilia emetica* root barks, and *Nauclea latifolia* stems were evaluated by diffusion and dilution methods on Mueller-Hinton agar (GMH) against 06 multidrug-resistant strains isolated from sick patients and 03 baseline strains. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The area of inhibition of the strains used induced by the extracts of *Nauclea latifolia*, *Parsia biglobosa,* and *Trichilia emetica*at 50 mg/mL is greater than that of the baseline antibiotics (cefoxitin, ceftriaxone, and imipenem). The active extracts showed a bactericidal profile with MICs and MBCs ranging from 3.125 mg/mL to 12.5 mg/mL.

INTRODUCTION

Infectious diseases are one of the leading causes of death worldwide. The World Health Organization (WHO) estimates that infectious diseases are responsible for 14 million deaths per year worldwide, and account for 43% of deaths in developing countries **(OMS, 1999).** Many antibiotics from conventional medicine have been developed to treat them. However, their misuse is at the root of the appearance of bacterial multi-resistance. This microbial resistance to these antibiotics has led researchers to investigate the use of natural remedies from the plant kingdom to address various diseases **(Guessennd et *al.*, 2009).** Research around the world has shown the effectiveness of several plants used in traditional medicine against microorganisms **(Sanogo et *al.*, 2006; Dupont et *al.*, 2006; Ndip et *al.*, 2007).** Therefore, four plants used in the center-east of Côte d'Ivoire for the treatment of various bacterial infections were selected for research. These are *Margaritaria discoidea* (Euphorbiaceae), *Nauclea latifolia* (Rubiaceae), *Parsia biglobosa* (Mimosaceae)and *Trichilia emetica* (Meliaceae).

Margaritaria discoidea and *Nauclea latifolia* are common species in tropical Africa **(Lemmens *and* al., 2008; Arbonier, 2002)**. *Parsia biglobosa* is common from Senegal through southern Sudan to northern Uganda **(Ajaiyeoba, 2002; Sacande & Clethero, 2007)**. As for *Trichilia emetica*, it is a fairly common species in Sudanese wooded and shrubby savannas**.** It is also present in Cameroon, Sudan, Upper and Middle Casamance, Eastern Senegal, and Uganda **(Kerharo & Adam, 1974; Malgras, 1992)**.

These four plants are used in many African countries to treat several infectious diseases, namely gonorrhea, diarrhea, skin infections, oral infections, and wounds **(Lemmens *et al.*, 2008; Sourabie, 1990; Sourabie *et al.*, 1995; Diallo, 2000)**. It is in this context that this study aims to investigate the antibacterial properties of the four above-mentioned plants against certain multi-resistant bacteria responsible for common infectious diseases.

1- EQUIPMENT AND METHODS

1.1.Plant matrix

It consists of trunk barks of *M. discoidea* (Md) and *P. biglobosa* (Pb), the stem of *N. latifolia* (NL), and root barks of *T. emetica* (Te). These plant species were selected on the basis of ethnobotanical surveys conducted among traditional therapists practicing in the Centre-East

of Côte d'Ivoire. They were then authenticated at the herbarium of the Centre National de Floristique (CNF) of the Félix Houphouët-Boigny University (Abidjan-Cocody). The harvest took place in July 2020 in Dabakala (Dabakala Department, Hambol Region). These plant species were cleaned, dried away from the sun for 2 days, then kept under air conditioning (18°C) for 7 days, and then stored in the oven (45°C) for 3 days. Powders obtained after grinding the plant matrix were used to prepare the extracts to be tested.

1.2.Bacterial strains

06 multi-resistant bacterial strains, including 02 *Escherichia coli* (1023 UB/21 CNRa, 2119 T/21 CNRa), 02 *Staphylococcus aureus* (1082 UB/21 CNRa, 1069 UB/21 CNRa) and 02 *Pseudomonas aeruginosa* (1120 UB/21 CNRa, 1129 TR/21 CNRa), from the *Unité des Antibiotiques, des Substances Naturelles et de la Surveillance des Microorganismes Anti-Infectieux (ASSURMI)* of the Department of Bacteriology and Virology of the Pasteur Institute of Côte d'Ivoire (IPCI) were used for the antibacterial tests. These strains were chosen because of their high frequency in several human infectious diseases.

1.3.Preparation of hydromethanolic extracts

15 g powder of each drug was macerated in 100 mL of MeOH (70%) for 24 hours under constant stirring. After removal of the solvent mixture (water + MeOH), the dry extract obtained is stored in a hermetically sealed jar in a refrigerator (4°C) (Zirihi et al., 2003). The crude hydromethanolic extracts obtained (Mb, Nl, Pb, and Te) were used to perform the antibacterial tests.

1.4. Antibacterial test of the different extracts

- Sterility testing of extracts:

0.1 g of the test extract in 10 mL of thioglycollate broth was incubated at 37°C for 24 h. The mixture was then seeded into a petri dish containing regular agar and incubated at 37°C for 24 hours. The substance is considered sterile if no colonies are visible on the agar plate **(Akers, 1985).**

- Preparation of the bacterial inoculum

The bacterial strains to be tested were grown in Petri dishes containing nutrient agar. After 18 hours of incubation at 37°C, bacterial suspensions were collected using a platinum loop, homogenized in 2 mL of sterile physiological water to obtain an optical density (OD) of 0.5 Mc Farland corresponding to a bacterial population of approximately 10^6 CFU/mL (standard condition). This inoculum was used to seed the MH agar plates for the test by tight streaks using a swab **(CASFM, 2020).**

- Bacterial inoculum count

Four successive 10^{th} dilutions from 10^{-1} to 10^{-4} were made from the initial inoculum. These dilutions and the starting inoculum were seeded in 5 cm strips on the different MH agar plates and incubated at 37°C for 24 hours. These Petri dishes constitute the A plates **(Guessennd *et al*., 2005).**

- Preparation of concentration ranges

An initial solution (50 mg/mL) of each extract was prepared. From this stock solution, a series of double dilutions using a geometric progression of reason ½ was performed to obtain 10 concentration ranges from 50 mg/mL to 0.09765 mg/mL (Konan, 2015).

- Sensitivity testing by solid-state diffusion

Each test extract at a concentration of 50 mg/mL was deposited into a well in an agar plate seeded and inoculated with a target bacterial strain. The mixture was incubated for 24 hours at 37°C and the diameter of the inhibition discs around each cup was measured with a caliper **(Wiegand *et al.*, 2007**; Guessennd et al., 2005). Sterile distilled water was used as a negative control. The positive control antibiotics cefoxitin (FOX), ceftriaxone (CRO), and imipenem (IPM) were chosen because of their usefulness in treating the conditions associated with the bacterial strains tested. This was repeated 3 times in a row and the average diameter was calculated.

- Determination of the minimum inhibition concentration (MIC) by liquid-state dilution

1 mL of the different concentration ranges of each plant extract is inserted into tubes numbered from highest to lowest concentration. Two other tubes containing 1 mL of sterile

distilled water and 2 mL of sterile physiological water are used as growth control (Tc) and sterility control (Ts) tubes respectively. Then 1 ml of the previously prepared 100 dilutions bacterial inoculum is added to each tube containing the different extracts and the growth control (Tc). All these tubes were incubated at 37°C for 24 h. The MIC, therefore, corresponds to the concentration of the first experimental tube from which no disorder is observed by the naked eye. This was repeated 3 times in a row.

- Determination of the minimum bactericidal concentration (MBC)

Using a calibrated loop (2 µL), the contents of tubes in which no cloudiness was observed with the naked eye for each extract, were seeded in 5 cm streaks on MH agar in a plate called plate B, starting with the MIC tube. This plate B was incubated for 24 hours at 37°C and then the number of colonies on the streaks of this plate was compared to that of the corresponding plate A. Thus, the first tube in which the number of germs on its streak is less than or equal to that of the 10^{-4} dilution will correspond to the MBC (lowest bactericidal concentration of a substance that leaves no more than 0.01% surviving germs).

1.5. Statistical analysis

The statistical analysis of the data was carried out using Excel 2007.

2- RESULTS AND DISCUSSION

2.1. Yields of hydromethanolic extractions

Maceration with 70% (v/v) methanol was used to extract the bioactive constituents of the plants. The hydromethanolic mixture is recommended for extraction as it provides the highest possible quantity of phytoconstituents **(Ladiguina et *al.*, 1983**; **Lagnika, 2005; Benkiki,2006)**. The yields from the maceration of the organs of the four plants studied are recorded in Table I.

Table I: Maceration yields

	Extracts			
	Md	**Nl**	**Pb**	**Te**
Powder weight (g)	15	15	15	15
Extracted weight (g)	0.19	0.39	0.20	0.42
Yield (%)	**12.66**	**26.00**	**13.33**	**28.00**

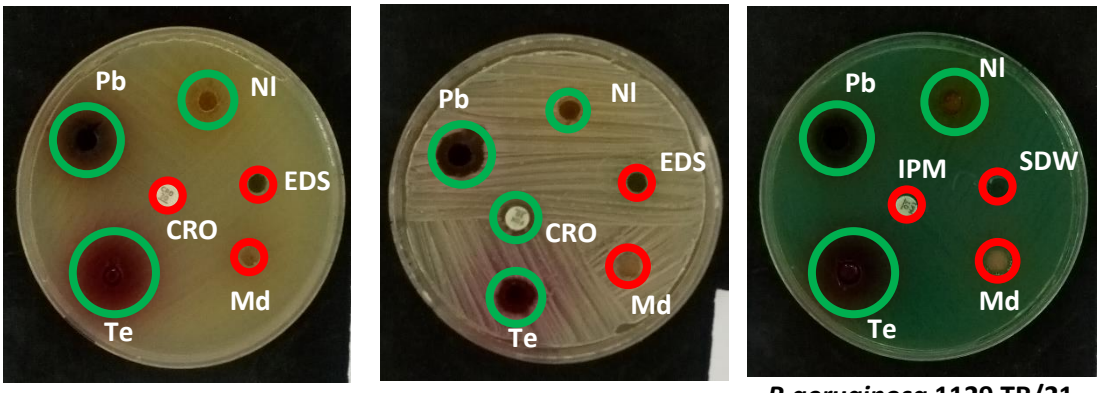
Yield values range from 12.66% (Md) to 28.00% (Te). These relatively high values are due, on the one hand, to the affinity of the solvent used regarding the phytomolecules and, on the other hand, to its polarity **(Dah-. Nouvléstounon *et al*, 2015)**. Methanol is a common solvent used to extract polyphenols. However, the presence of water also increases the permeability of plant tissues and fosters the phenomenon of broad dissemination in the extraction stage **(Moure *et al.*, 2000; Trabelsi *et al.*, 2010; Arimboor et Arumughan, 2011)**.

2.2. Sterility testing of plant extracts

The sterility test carried out showed that all plant extracts were free of contamination as there were no colonies in the different agar plates after 24 hours.

2.3. Solid-state antibacterial activity

The results of the sensitivity of the bacteria to hydromethanolic extracts of the four plants tested and the baseline antibiotics are presented in Figure 1 and summarized in Tables II, III, and IV.



***E.coli* 1023 UB/21 CNRa** **S.aureus 1069 UB/21** ***P.aeruginosa* 1129 TR/21**

Figure 1: Sensitivity testing of the extracts for the study and the antibiotics against bacteria

: Sensitive : Resistant

Table II: Diameters (mm) of the inhibition zones of *Escherichia coli* by the plant extracts and the baseline antibiotic

Strains	**Average diameter (D) of bacterial inhibition zones of 50 mg/mL extracts, antibiotic and sterile distilled water**					
Escherichia Coli	**Md**	**Nl**	**Pb**	**Te**	**CRO**	**SDW**
E. coli 1023 UB/21CNRa	06±0.0	**9.27±0.13**	**14.43±0.12**	**15.9±0.1**	**06±0.0**	06±0.0
E. coli 2119 T/21 CNRa	06±0.0	06±0.0	**10.4±0.35**	**13.2±0.2**	**10.13±0.08**	06±0.0

Md, Nl, Pb, and Te: methanolic extracts of *Margaritaria discoidea*, *Naucela latifolia*, *Parsia biglobosa*, and *Trichilia emetica* respectively; CRO: Ceftriaxone; SDW: Sterilized distilled water; UB: Cocody Academic Hospital; T: Treichville Academic Hospital; CNRa: National antibiotic Reference Centre.

Table III: Diameters (mm) of the inhibition zones of Staphylococcus aureus by the plant extracts and the baseline antibiotic.

Strains	**Average diameter (D) of bacterial inhibition zones of 50 mg/mL extracts, antibiotic and sterile distilled water**					
Staphylococcus aureus	**Md**	**Nl**	**Pb**	**Te**	**FOX**	**SDW**
S. aureus 1082 UB/21 CNRa	06±0.0	06±0.0	**13.1±0.5**	**10.1±0.41**	**06±0.0**	06±0.0
S. aureus 1069 UB/21 CNRa	06±0.0	**10.1±0.45**	**12.1±0.05**	**11.1±0.45**	**09±0.0**	06±0.0

Md, Nl, Pb, and Te: methanolic extracts of *Margaritaria discoidea*, *Nauclea latifolia*, *Parsia biglobosa*, and Trichilia emetica respectively; FOX: Cefoxitin; SDW: Sterilized distilled water; UB: Cocody Academic Hospital; CNRa: National antibiotic Reference Centre.

Table IV: Diameters (mm) of the inhibition zones of Pseudomonas aeruginosa by the plant extracts and the baseline antibiotic.

Strains	**Average diameter (D) of bacterial inhibition zones of 50 mg/mL extracts, antibiotic and sterile distilled water**					
Pseudomonas aeruginosa	**Md**	**Nl**	**Pb**	**Te**	**IPM**	**SDW**
P. aeruginosa 1120 UB/21 CNRa	06±0.0	06±0.0	**10.53±0.26**	**9.33±0.18**	**06±0.0**	06±0.0
P. aeruginosa 1129 TR/21 CNRa	06±0.0	**12.93±0.22**	**14.43±0.12**	**15.8±0.2**	**06±0.0**	06±0.0

Md, Nl, Pb, and Te: methanolic extracts of *Margaritaria discoidea*, *Naucela latifolia*, *Parsia biglobosa*, and *Trichilia emetica* respectively; IPM: imipenem; SDW: sterilized distilled water; UB: Cocody Academic Hospital; TR: Treichville Academic Hospital; CNRa: National antibiotic Reference Centre.

The diameters of the inhibition zones of bacterial strains by plant extracts vary from one strain to another depending on the extract used. Some extracts show antimicrobial activity while others do not. The strain is resistant to the substance when the diameter of its inhibition zone is less than 8 mm, sensitive when it is between 9 and 14 mm, very sensitive when it is between 15 and 19 mm, and extremely sensitive when it is over 20 mm **(Ponce *et al.*, 2003).**

The extracts of *Parkia biglobosa* and *Trichilia emetica* showed activity against all bacterial strains used with the diameters of the inhibition zones between 9.33±0.18 and 15.9±0.1mm (Table II, III, and IV). The extract of the stems of *Nauclea latifolia* was only active against *Escherichia Coli*1023 UB/21CNRa, *Staphylococcus aureus* 1069 UB/21 CNRa et *Pseudomonas aeruginosa* 1129 TR/21 CNRa with diameters of inhibition zone of 9.27±0.13; 10.1±0.45 and 12.93±0.22 mm respectively. Furthermore, the diameters of inhibition zones shown by the *P. biglobosa* and *T. emetica* extracts are significantly larger than those shown by the *N. latifolia extract.*

This antibacterial activity would be partly due to the presence of antibacterial substances and, more particularly, to phenolic compounds, whose remarkable presence has been reported in *N. latifolia*, *P. biglobobosa*, and *T. emetica* by several authors **(Uwah *et al.*, 2010; Vieira *et al.*, 2013; Ouattara L.H. *et al.*, 2016)**. Phenolic compounds have very good antibacterial activity **(Bruneton, 1993; Wollgast et *al.*, 2000; Sannomiya et *al.*, 2005; Konaté *et al.*,**

2015). As for *Margaritaria discoidea*, its extract was inactive on all strains used with diameters of inhibition zones below 8 mm. This can be explained by the fact that the root barks of *M. discoidea* contain fewer phenolic compounds. Indeed, the dosage of polyphenols performed by **Ouattara L.H. *et al.* (2016)** revealed that their quantity in*M. discoidea* (2444.35±0.01 µgEAG/g) is significantly lower than that found in *N. latifolia* (3505.02±0.02 µgEAG/g), *P. biglobosa* (8805.93±0.01 µgEAG/g), and *T. emetica* (3640.15±0.01 µgEAG/g).

The baseline antibiotics (ceftriaxone and cefoxitin) had mild effects on *E. coli* 2119 T/21 CNRa and *S. aureus* 1069 UB/21 CNRa, respectively, with diameters of inhibition zone of 10.13±0.08 and 09±0.0 mm, respectively. Compared to the active plant extracts, they showed much smaller diameters of the inhibition zones. In addition, imipenem (the third baseline antibiotic) had no effect on the strains tested. These results confirm the resistance frequently observed in certain pathological bacterial strains to conventional antibiotics **(Guessennd *et al.*, 2009)** and justify the orientation of a large amount of research on plant-based antibiotics **(Dupont et *al.*, 2006; Sanogo et *al.*, 2006); Ndip et *al.*, 2007; Guessennd *et al.*, 2009).**

2.4- Liquid-state antibacterial activity

The MICs and MBCs of the extracts against the bacterial strains were determined, e.g. their determination against *S. aureus* is shown in Figure 2.

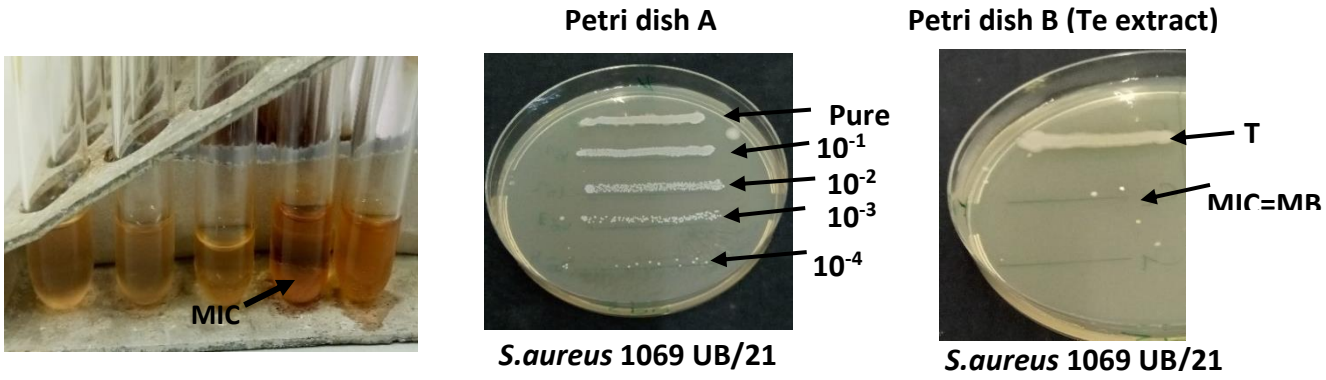


Figure 2: Determination of the MICs and MBCs.

Tables V to VII represent the MIC/MBC ratios determined for each plant studied against all the selected bacterial strains.

Table V: Antibacterial parameters of plant extracts against *Escherichia coli*

Strains	Extracts	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
E. coli 1023 UB/21CNRa	Nl	12.5	12.5	1	**Bactericide**
	Pb	3.125	3.125	1	**Bactericide**
	Te	3.125	3.125	1	**Bactericide**
E. coli 2119 T/21 CNRa	Pb	6.25	6.25	1	**Bactericide**
	Te	12.5	12.5	1	**Bactericide**

Table VI: Antibacterial parameters of plant extracts against *Staphylococcus aureus*

Strains	Extracts	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
S. aureus 1082 UB/21 CNRa	Pb	3.125	3.125	1	**Bactericide**
	Te	3.125	3.125	1	**Bactericide**
S. aureus 1069 UB/21 CNRa	Nl	12.5	12.5	1	**Bactericide**
	Pb	3.125	3.125	1	**Bactericide**
	Te	12.5	12.5	1	**Bactericide**

Table VII: Antibacterial parameters of plant extracts against *Pseudomonas aeruginosa*

Strains	Extracts	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
P. aeruginosa 1120 UB/21 CNRa	Pb	12.5	12.5	1	**Bactericide**
	Te	12.5	12.5	1	**Bactericide**
P. aeruginosa 1129 TR/21 CNRa	Nl	3.125	3.125	1	**Bactericide**
	Pb	3.125	3.125	1	**Bactericide**
	Te	3.125	3.125	1	**Bactericide**

The MBC/MIC ratio of an extract indicates the nature of the antibacterial activity, when MBC/MIC ≤ 2, the extract is bactericidal, and if MBC/MIC > 2, it is bacteriostatic. (**Fauchere & Avril, 2002)**. From this point of view, the hydromethanolic extracts of *Parkia biglobosa* and *Trichilia emetica* are bactericidal against all the multi-resistant strains used. Concerning *Nauclea latifolia*, its extract is only bactericidal against *E. coli* 1023

UB/21CNRa**,** *S. aureus* 1069 UB/21 CNRA, and *P. aeruginosa* 1129 TR/21 CNRa (**Table V, VI, and VII**).

The antibacterial activities found in *N. latifolia*, *P. biglobosa*, and *T. emetica* conform with research on *N. latifolia* from Burkina Faso (**Kaboré *et al.*, 1995),** on the Nigerian species of P. *biglobosa* (**Aewale *et al.*, 2012)** and on the roots extracts of *T. emetica* (**Germano *et al.*, 2005)**.

CONCLUSION

In order to provide a rational explanation for the use of *Margaritaria discoidea*, *Nauclea latifolia*, *Parsia biglobosa*, and *Trichilia emetica* in traditional medicine in the treatment of certain infectious diseases, the antibacterial activity of their hydromethanolic extracts was carried out.

Liquid-state tests of the said extracts revealed that the hydromethanolic extracts of *Nauclea latifolia*, *Parsia biglobosa*, and *Trichilia emetica* exhibit an antibacterial effect on most of the multi-resistant bacteria used. They showed higher activity than the baseline antibiotics used, which no antibacterial activity on the strains had used. This should create particular interest for extracts of *N. latifolia*, *P. biglobosa*, and *T. emetica* being potentially suitable for the development of drugs against bacterial infections.

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